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1.1 INTRODUCTION

Optical surface texture measurement and characterization of dental hard tissues is becoming increasingly used within dentistry as a method for detecting and quantifying early enamel damage resulting from common oral pathologies such as dental erosion [1, 2]. When quantifying the micro-texture of enamel surface damage, the earliest signs of wear occur at the scale of an enamel prism. Accordingly, recent research into nanometer scale surface changes of polished human enamel using confocal laser microscopy concluded that optimal characterization of acid mediated surface texture changes requires surface metrology instrumentation with lateral resolution less than 2.5 μm [3]. Chromatic Confocal profilometry is industry standard for optical surface metrology and is specifically recommended by the ISO 25178 international standard for non-contact 3D metrology [4, 5]. However, the lateral resolution of chromatic confocal profilometers is limited by the sensor spot diameter varying from up to 24 microns and also by lateral mechanical scanning which introduces measurement noise during the movement of the x,y stage [6]. In contrast the lateral resolution of confocal laser scanning microscopy is typically in the order of 100 nm and no mechanical scanning is required [7]. Therefore, as confocal chromatic profilometry is increasingly recommended for dental research into the topography and texture of natural and biological materials [2, 8], there is a need to understand the measurement performance of surface metrology instrumentation operating at supra-micrometer level resolutions.

There are many potential sources of measurement error which can undermine certainty of measurement, including instrument bias, mechanical or optical changes due to ageing, wear, or other kinds of drift, poor readability and environmental or electrical noise; as well as specimen issues such as dimensional instability, or other operator, process or environmental derived errors [9]. In the case of surface metrology for mineralized tissue and dental materials research applications, potential sources of error include those associated with the fundamental operating principles of the profilometer [10], as well as potential dimensional instability from enamel sample dehydration and rehydration during measurement [11]. One method of understanding the quality of a measurement system for a given application is to assess the uncertainty of measurement [9]. Uncertainty is a quantification of the doubt about the measurement result and is used in engineering industry to identify, quantify, and characterize each independent variable contributing errors in the measurement process, in order to evaluate and reduce these errors thus improving measurement quality.

The aim of this study was to investigate the measurement performance of a chromatic confocal profilometer for quantification of acid-mediated surface texture changes in human enamel. The objectives of this study were a) to quantify the measurement uncertainty associated with chromatic confocal profilometry of human enamel undergoing erosive surface damage and b) to optimise chromatic confocal profilometry for characterisation of erosive surface texture changes in natural and polished enamel *in vitro*.

1.2 MATERIALS AND METHODS

A chromatic confocal sensor (STIL OP350VM, France) mounted on a non-contact profilometer (Xyris 4000, Taicaan, Southampton, UK) operating with 3.5 μm lateral resolution and 10 nm vertical resolution was used under carefully controlled conditions throughout this study. As shown in Figure 1, the optical principles of the sensor involved passing polychromatic white-light (a) through a chromatic lens (b) to generate a continuum of monochromatic light located on the optical axis. Samples surfaces (c), located within the optical z range below the sensor at a specified position on the x,y stage, scattered the incident light beam back through the chromatic lens (b), via a beamsplitter (d) to a pinhole (e), which filtered the single reflected wavelength (λ) representing a set distance from the

lens. A spectrometer (f) thus allocated the sample surface z position according to the detected wavelength of peak intensity (g). The stage then moved to the next x, y position in a raster pattern and thus the entire sample surface was scanned [7]. Resulting topography data were exported to surface metrology software (MountainsMap® V7.2; Digital Surf, Besançon, France), validated to ISO 3D surface metrology standards and all measurements were conducted by a single operator [4].

For the uncertainty analysis, potential sources of measurement error were identified following advice from dimensional metrologists at the UK's National Measurement Institute (National Physical Laboratory, Teddington, UK) and these were systematically investigated using calibration artefacts and the results were propagated using an uncertainty budget following good practice metrology guidelines [9, 12, 13]. Firstly, a calibrated optical flat (National Physical Laboratory, Teddington, UK) was used to quantify measurement noise added to the output signal occurring during the normal use of the instrument and flatness deviations which indicates the quality of the areal reference of the instrument. Three repeated 5 mm by 3 mm areas of the optical flat were scanned across five positions of the x, y stage; four in the peripheral corners and one in the central x,y position. Measurement noise was quantified using the maximum root mean square value of the scale limited surface (S_q) and flatness deviations were quantified using the measured maximum height of the scale limited surface (S_z) [4].

Lateral (x, y) linearity errors were quantified across 5 mm of a calibrated chrome-on-quartz linear scale (SC6 Lateral Scale, Microscopy Optical Dimensional Standard, National Physical Laboratory, UK) with 10 μm nominal line width and 100 μm nominal pitch [14]. The scale was positioned in x and y orientations and scanned three times per axis. Resulting 3D profile data were aligned parallel to x or y axes, following which a mean 2D profile was extracted and the raw 2D data were exported to spreadsheet software (Microsoft Excel 2010, Microsoft Office). The central point of each line on the lateral scale was identified thus allowing the mean (SD) difference between the nominal position of the centre of each line on the lateral scale and the measured position of the centre of each line on the lateral scale to be expressed across the x and y lateral axes and the maximum linearity error (μm) was calculated for each axis.

Vertical linearity errors were quantified along the z axis using calibrated glass 0.3 μm , 2.97 μm , 17 μm and 30 μm step height standards (Type A1 reference standard, Taylor Hobson Ltd, Leicester, United Kingdom). Each step height reference standard was placed onto the central position in the x,y stage and scanned at three positions across the z stage (high at + 12.5 mm, middle and low at -12.5 mm), in order to quantify the contribution of non-linearity on the vertical scale [15]. The mean 3D step height was calculated by comparing the mean height of the central third of the bottom of the step with the mean height of the central third of the reference plane [16]. The mean (SD) differences between the nominal step height and the measured result were calculated and the maximum linearity error (μm) on the vertical axis was determined.

Sound human molars were collected under ethical agreement (REC: 12/LO/1836). Ten samples were polished to 0.4 μm flatness tolerance and pre-eroded with 0.3% citric acid following previously published protocols [17], to create step heights with depth ranging from 3- 30 μm . The impact of dimensional instability caused by enamel sample dehydration on the measurement uncertainty was quantified by serial step height measurement of the enamel samples during repeated dehydration and rehydration cycles (1 cycle = 120 minutes) in artificial saliva [18]. The mean percent change (%) in measured step height during dehydration/ rehydration cycles calculated and the maximum dimensional instability (μm) was determined. Finally, all standard measurement uncertainty (u) contributions in μm were combined following a Type B uncertainty evaluation and the overall

uncertainty was expressed as the combined standard uncertainty (u_c) as \pm in μm following metrology good practice guidelines [9, 12].

Using the resulting information, an optimized measurement protocol was developed for surface texture measurement of natural human enamel samples undergoing enamel erosion from a dietary acid (Sainsbury's Basic Orange Juice, London, UK) with pH 3.2 and titratable acidity 41.3 mmol OH/L. 30 polished and 30 unpolished enamel samples were randomly allocated into three groups ($n=10/\text{group}$). Group one underwent three cycles of five minutes' immersion at 62 rpm agitation using an orbital shaker (Stuart Scientific, Mini Orbital Shaker S05, Bibby). Group two underwent three cycles of ten minutes' erosion and group three underwent three cycles of 15 minutes' erosion. Each sample was scanned before and after erosion using five $200\text{ }\mu\text{m} \times 200\text{ }\mu\text{m}$ areas systematically selected from the centre of the sample, scanned with a $4\text{ }\mu\text{m}$ scanning interval. For both groups, the surface image was levelled and a $25\text{ }\mu\text{m}$ Gaussian filter applied to isolate the 3D roughness (S_a) data following previous protocols [3]. In addition, representative qualitative analysis of enamel surface textural changes was carried out using environmental Scanning Electron Microscopy (Phenom ProX desktop SEM, Phenom-World BV, The Netherlands) at $\times 1100$ magnification (0.06 mm^2).

The individual errors from the flatness deviations, noise, x,y,z non-linearities, software errors and enamel and dentine shrinkage were quantified and the measurement uncertainty was calculated using a Type B uncertainty evaluation [9, 12]. Each standard uncertainty (u) was calculated as $u = \frac{a}{\sqrt{3}}$, where a is the half-width between the upper and lower limits of each individual contribution to the uncertainty budget in μm . The standard uncertainties were then combined by calculating the root sum of the squares of all the uncertainties and the result represented the Standard Combined Uncertainty (u_c) equivalent to 'one standard deviation' around the measurement result and therefore expressed as $\pm\text{ }\mu\text{m}$ [9].

For the surface texture measurement, the sample size was based upon previous studies [19]. Kolmogorov-Smirnov, Shapiro-Wilk tests and histogram plots were used to assess normality. Data were non-normally distributed therefore Independent Kruskal Wallis one way analysis on ranks were used for group comparisons of the surface texture at baseline and after erosion. Paired Mann-Whitney Rank Sum and post-hoc Dunn's tests to compare groups individually before erosion versus after erosion. SPSS and Sigmaplot were used to analyse the data and statistical significance was set at $p < 0.05$.

1.3 RESULTS

Measurement of the optical flat revealed the profilometer had maximum S_q noise error of $0.08\text{ }\mu\text{m}$ S_z flatness error of $0.49\text{ }\mu\text{m}$ across the $3\text{ mm} \times 5\text{ mm}$ area, as shown in Figure 2. Quantification of the x, y nonlinearities revealed that the maximum x axis error was $10.22\text{ }\mu\text{m}$ and y axis error $12.14\text{ }\mu\text{m}$ across the 5 mm lateral scale, as shown in Figure 3. Quantification of the vertical (z) scale using the $0.3\text{ }\mu\text{m}$ to $30\text{ }\mu\text{m}$ step heights revealed that the linearity errors reached a maximum of 40 nm , as shown in Figure 4. The impact of dimensional instability caused by enamel sample dehydration/rehydration was quantified as a maximum of 0.03% , which represented 9 nm for the largest sample step-height of $30\text{ }\mu\text{m}$. When these values were combined into the uncertainty budget, the Standard Combined Uncertainty (u_c) of measurement using the chromatic confocal profilometer and the metrology software for surface metrology of enamel was $\pm 0.28\text{ }\mu\text{m}$.

The optimised $0.04\text{ }\mu\text{m}^2$ 3D roughness measurement protocol, shown in Figure 5, revealed a statistically significant increase for all three erosion times for polished enamel, the median (IQR) 3D surface roughness (S_a) of polished enamel samples undergoing erosion measurements ($P < 0.001$). For 15 minutes erosion, the median (IQR) S_a increased from 0.08 (0.10) μm at baseline to 0.26 (0.02) μm

($P < 0.001$); for 30 minutes erosion from 0.15 (0.11) μm at baseline to 0.25 (0.07) μm after erosion and for 45 minutes erosion from 0.10 (0.08) μm at baseline which significantly increased to 0.27 (0.04) μm after erosion ($P < 0.001$). In contrast, the natural unpolished samples undergoing erosion, showed reductions in median (IQR) Sa roughness from 0.65 (0.30) μm to 0.49 (0.35) μm after 15 minutes erosion and from 0.48 (0.38) μm to 0.44 (0.2) μm after 30 minutes erosion, however these values were not statistically significantly different ($P > 0.05$), until the natural enamel samples underwent 45 minutes erosion, when the reductions in the median (IQR) Sa roughness of natural enamel samples from 0.50 (0.29) μm at baseline to 0.42 (0.14) μm after erosion became statistically significant ($P < 0.05$).

Representative SEM images shown in Figure 6 and revealed the presence of minimal surface textural features at baseline for the polished enamel except for residual scratch marks from the polishing regime. After 15, 30 and 45 minutes of erosion, typical demineralised prismatic pattern appearance is evident, where the centres of the enamel prisms have been dissolved and the adjacent interprismatic areas are raised. For the natural enamel samples Figure 7 reveals variation in enamel structure with identifiable features including perikymata and a few exposed enamel prisms at baseline. After 15 and 30 minutes of erosion there was an increase in identifiable eroded prismatic features however the overall surface remained intact. After 45 minutes of erosion there is evidence of structural breakdown and increased preponderance of erosive prismatic features.

1.4 DISCUSSION

The estimation of the measurement uncertainty was carried out in order to determine the main sources of measurement errors during erosive tooth wear measurement using the chromatic confocal profilometer and surface metrology software. The overall quality of measurement was expressed via the combined standard uncertainty, as outlined in the "Guide to the Expression of Uncertainty in Measurement" (GUM) [12]. This involved the creation of an 'uncertainty budget' which states the corresponding variance of the quantity value from each potential source of error, which in this case involved measurement error from flatness deviations and measurement noise, non-linearities in the x,y and z and from dimensional instability due to sample shrinkage during dehydration and rehydration. This exercise revealed that the greatest contributions to the measurement uncertainty were from flatness errors of up to almost half a μm . In Figure 2, the flatness deviation can be seen in the 3D profile of the waviness of the surface characterised by the waviness of the optical flat measurement. This revealed errors caused by the form of the drive screw during physical movement of the x,y stage during raster scanning and resulted in regular 'hills' and 'dales' with a maximum height of the scale limited surface (S_z) of 0.49 μm . In addition, the 3D roughness profile shown in Figure 2, revealed noise errors from the movement of the ball bearings supporting the stage represented by crisscrossed patterns running parallel to the x and y axis with a maximum root mean square height of the scale limited surface (S_q) 0.04 μm . Measurement of the NPL optical dimensional standard lateral scale shown in Figure 3 revealed errors during lateral movement of the x,y stage, driven by the linear encoder within the motion control system, resulting in a peak measurement error of 12 μm in the centre of the 5 mm scale, however there was zero error at the start and end of the scale. This suggested that the motion controller started and finished x,y scanning with almost perfect precision and accuracy, however in the centre of the scale, errors from the linear encoder cumulated. As, these errors accumulated across larger distance therefore for the enamel roughness measurements a maximum x,y distance of 200 μm was chosen. Accordingly, non-linearities in the lateral scale were not considered to be a major source of measurement uncertainty in this present study as the surface texture measurement employed comparisons from points very close to each other. Additionally, the contribution to the uncertainty budget from z axis non-linearities was negligible at a maximum of 40 nm adds confidence to the measurement as the surface texture parameters used in this study were

all amplitude parameters (i.e. based on relative z comparisons of neighbouring x, y data) [4]. Similarly, errors caused by dimensional instability due to rehydration / dehydration were found to have negligible contribution to the uncertainty budget, indeed this may be more relevant for dentine sample measurement as opposed to enamel sample measurement [11].

Therefore, subsequent measurement of the 3D surface texture of natural and polished enamel samples was optimized both by ensuring that the small scan x, y scan area of 200 μm x 200 μm minimized any impact from the flatness deviations or x,y linearity errors as well as by filtering of any textural details greater than 25 microns ; chosen as approximately 5 times an enamel prism diameter [2, 3]. This allowed maximal utilization of high z resolution of the chromatic confocal sensor thus aiding measurement of 3D surface texture parameters based on calculation of the z amplitude of the roughness profile, such as Sa [4]. The measurement device was thus able to detect statistically significant differences in the surface texture, corroborated using SEM imaging. Polished enamel became significantly rougher after 15, 30 and 45 minutes of erosion in orange juice ($P<0.001$), respectively increasing from 0.08 (0.10) μm , 0.15 (0.11) μm and 0.10 (0.08) μm to 0.26 (0.02) μm , 0.25 (0.07) μm and 0.27 (0.04) μm . Whereas, natural enamel became significantly smoother after 45 minutes of erosion in orange juice with median (IQR) roughness decreasing from 0.50 (0.29) μm at baseline to 0.42 (0.14) μm after erosion ($P<0.05$). However, there was no statistically significant difference in Sa roughness after 15 or 30 minutes of erosion in orange juice.. For 15 and 30 minutes of erosion of natural enamel there were no significant changes however this corresponds with previous research suggesting that natural surfaces require increased erosion times before quantitative changes can be detected [20]. Previous studies have also suggested that natural enamel is less susceptible to the effects of acid induced erosion compared to polished enamel through examining SEM images before and after erosion and measuring tissues loss of both natural enamel and polished enamel samples [21]. The clinical relevance of this present study is difficult to discuss as there remain very few erosion studies investigating the 3D roughness of natural enamel. The unpolished enamel findings of this present study concur with previous research [3], however in the only similar study of its kind, Hara et al [22] were unable to detect Sa surface roughness changes in natural enamel after erosion, whereas this present study has found that Sa reduces changes as the erosion progresses. Removal of the aprismatic layer in polished enamel samples is thought to reduce the resistance to erosion which makes it challenging to characterize changes in Sa roughness of natural enamel samples. Therefore, it is necessary to develop methods of measuring natural enamel *in vitro* before these methods can be applied to an *in vivo* setting and measurement apparatus must have an adequate resolution to detect the subtle changes in the lesser affected natural enamel as well as the more obvious changes in polished samples.

The Chromatic Confocal Profilometer used in this study had measurement performance capable of detecting these changes, suggesting that the level of resolution required to identify textural changes in enamel is less than previously predicted, however further work is required to apply these findings to *in vivo* erosion states, as the influence of biological variable such as the enamel pellicle will modify the measurement of enamel surface texture [23]. Therefore, when attempting to carry out high quality measurement it is important to consider all possible sources of uncertainty, in order to develop optimal strategies for the specific measurement application, especially if the measurement technique requires reliable characterisation of the earliest signs of erosive enamel damage *in vivo*.

1.5 CONCLUSION

Assessment of measurement uncertainty during 3D surface texture measurement on natural enamel samples revealed the largest contribution to measurement uncertainty was from flatness deviations which resulted in a combined measurement uncertainty of $\pm 0.28 \mu\text{m}$. However, by carrying out surface

roughness measurements across small areas of natural enamel, optical profilometers with lateral resolution of 3.5 μm are capable of reliably detecting 3D surface roughness changes to natural enamel from acid erosion.

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1.7 DECLARATION OF INTERESTS

None.

1.8 CONFLICTS OF INTEREST

None.

1.9 DECLARATION OF FUNDING

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REFERENCES

1. Las Casas, E., et al., *Enamel wear and surface roughness characterization using 3D profilometry*. Tribology International, 2008. **41**(12): p. 1232-1236.
2. Austin, R.S., F. Mullen, and D.W. Bartlett, *Surface texture measurement for dental wear applications*. Surface Topography: Metrology and Properties, 2015. **3**(2): p. 023002.
3. Austin, R.S., et al., *Confocal laser scanning microscopy and area-scale analysis used to quantify enamel surface textural changes from citric acid demineralization and salivary remineralization in vitro*. Dental Materials, 2015 (DOI:10.1016/j.dental.2015.11.016).
4. ISO/DIS, 25178, in *Geometrical product specifications (GPS)—Surface texture: area*. 2011, International Organization for Standardization: Geneva.
5. Blateyron, F., *Chromatic Confocal Microscopy*, in *Optical Measurement of Surface Topography*, R. Leach, Editor. 2011, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 71-106.
6. Ruprecht, A.K., et al. *Chromatic confocal detection for high-speed microtopography measurements*. 2004.
7. Leach, R., *Fundamental principles of engineering nanometrology*. 2010.
8. Zheng, J., et al., *On the friction and wear behaviour of human tooth enamel and dentin*. Wear, 2003. **255**: p. 967-974.
9. Bell, S., *A beginner's guide to uncertainty of measurement NPL Good Practice Guide No 11*. Teddington, Middlesex: National Physics Laboratory, 2010.
10. Leach, R.K., *Some basics of measurement*, in *Fundamental principles of engineering nanometrology*. 2010, William Andrew ; Elsevier Science: Oxford; Amsterdam. p. 5-34.

- 269 11. Ganss, C., A. Lussi, and J. Klimek, *Comparison of calcium/phosphorus analysis, longitudinal*
270 *microradiography and profilometry for the quantitative assessment of erosive*
271 *demineralisation*. Caries research, 2005. **39**(3): p. 178-184.
- 272 12. Joint Committee for Guides in Metrology, *Evaluation of measurement data — Guide to the*
273 *expression of uncertainty in measurement*, in GUM 1995 with minor corrections. 2008.
- 274 13. Giusca, C., L., et al., *Calibration of the scales of areal surface topography-measuring*
275 *instruments: part 1. Measurement noise and residual flatness*. Measurement Science and
276 Technology, 2012. **23**(3): p. 035008.
- 277 14. National Physical Laboratory. *NPL Optical Dimensional Standard For Vision Machines &*
278 *Microscopy* 2016 [cited 2016 6th December]; Available from:
279 http://www.npl.co.uk/upload/pdf/ods_photomask.pdf.
- 280 15. Giusca, C.L., R.K. Leach, and F. Helery, *Calibration of the scales of areal surface topography*
281 *measuring instruments: part 2. Amplification, linearity and squareness*. Measurement Science
282 and Technology, 2012. **23**(6): p. 065005.
- 283 16. ISO, B., 3274. *Geometrical product specifications (GPS)–surface texture: profile method:*
284 *nominal characteristics of contact (stylus) instruments*. 1996, London: British Standards
285 Institution.
- 286 17. Mistry, M., et al., *Effect of Model Variables on in vitro Erosion*. Caries Research, 2015. **49**(5):
287 p. 508-514.
- 288 18. Eisenburger, M., et al., *Effect of time on the remineralisation of enamel by synthetic saliva*
289 *after citric acid erosion*. Caries Res, 2001. **35**(3): p. 211-5.
- 290 19. Austin, R., et al., *The effect of increasing sodium fluoride concentrations on erosion and*
291 *attrition of enamel and dentine in vitro*. Journal of Dentistry, 2010. **38**(10): p. 782-787.
- 292 20. Chew, H.P., et al., *Measuring Initial Enamel Erosion with Quantitative Light-Induced*
293 *Fluorescence and Optical Coherence Tomography: An in vitro Validation Study*. Caries
294 Research, 2014. **48**(3): p. 254-262.
- 295 21. Meurman, J.H. and R.M. Frank, *Progression and Surface Ultrastructure of in vitro Caused*
296 *Erosive Lesions in Human and Bovine Enamel*. Caries Research, 1991. **25**(2): p. 81-87.
- 297 22. Hara, A.T., et al., *Dental Surface Texture Characterization Based on Erosive Tooth Wear*
298 *Processes*. Journal of Dental Research, 2016. **95**(5): p. 537-542.
- 299 23. Moazzez, R.V., et al., *Comparison of the possible protective effect of the salivary pellicle of*
300 *individuals with and without erosion*. Caries Res, 2014. **48**(1): p. 57-62.